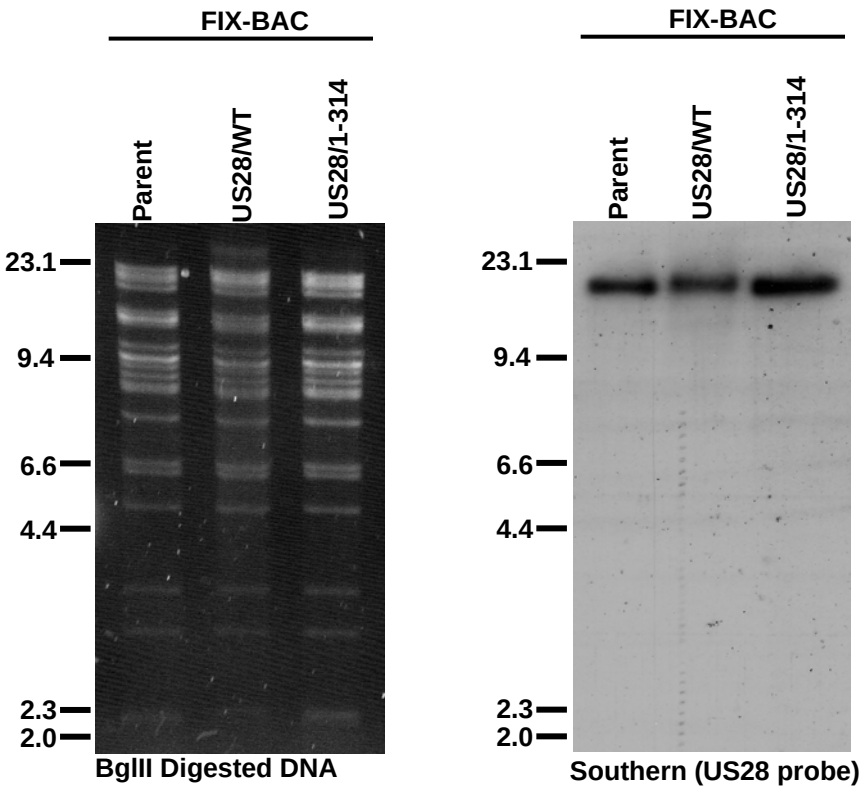
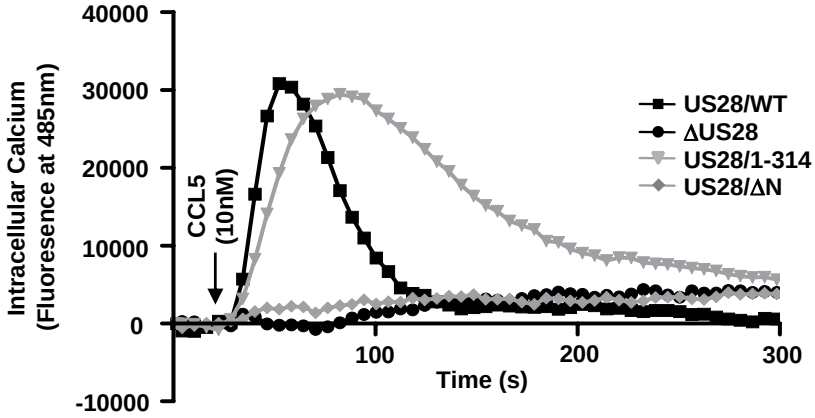
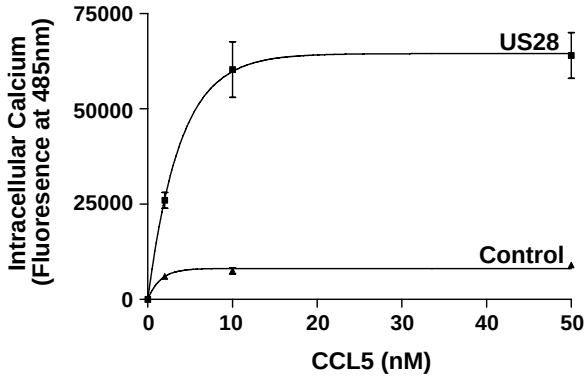
Stropes et. al. Supplemental Figure 1

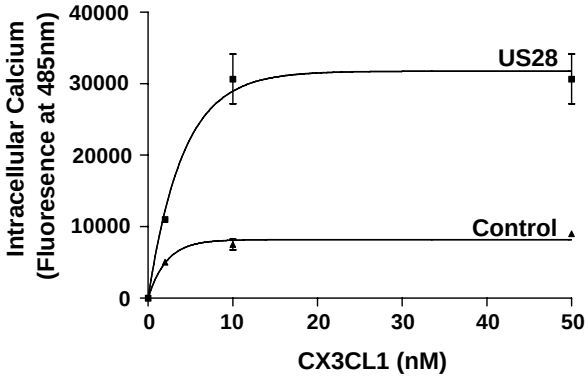


Stropes et. al. Supplemental Figure 2

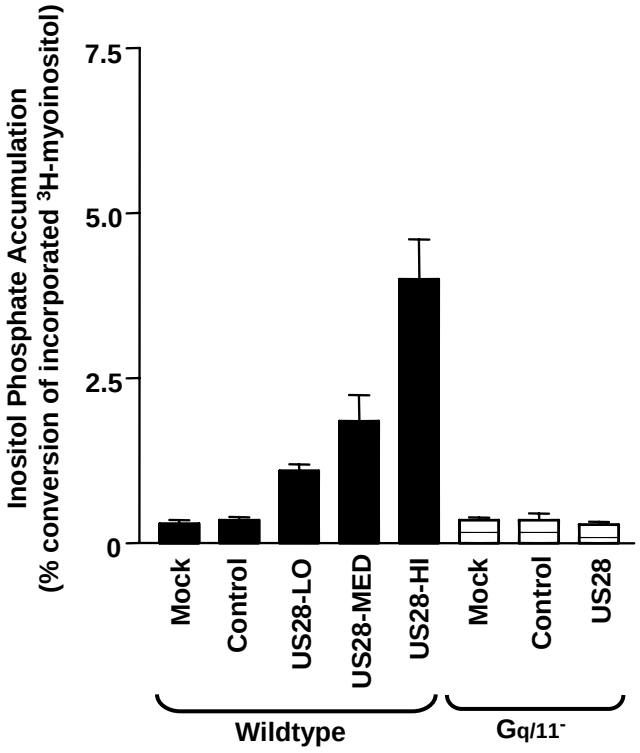


Stropes et. al. Supplemental Figure 3

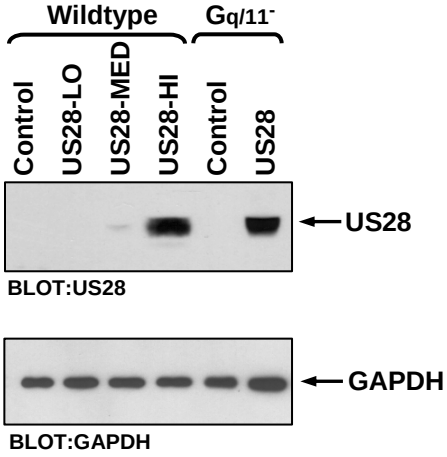




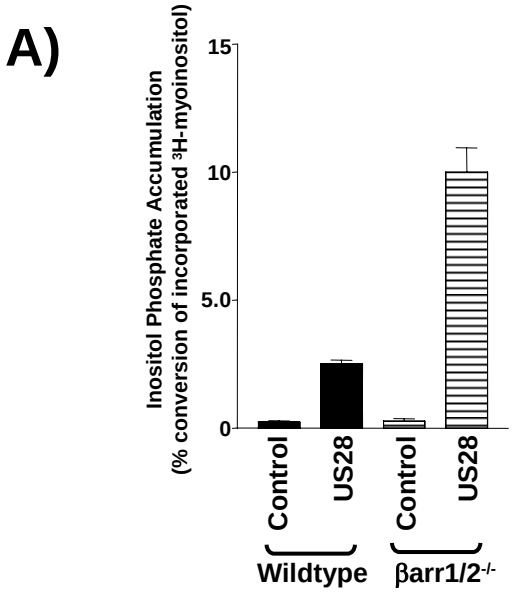
A)

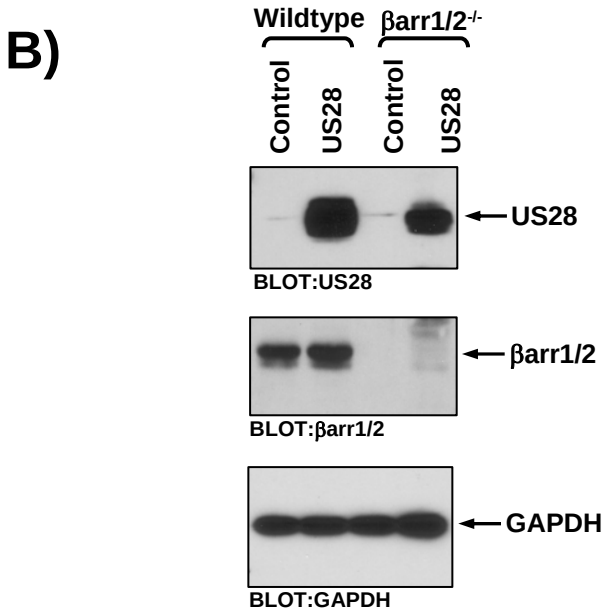

Inositol Phosphate Accumulation
(% conversion of incorporated ³H-myoinositol)
7.5
5.0
2.5
0
Mock
Control
US28-LO
US28-MED
US28-HI
Mock
Control
US28
Wildtype
Gq/11-

B)


Wildtype
Gq/11-
Control
US28-LO
US28-MED
US28-HI
Control
US28
US28
BLOT:US28
GAPDH
BLOT:GAPDH

Stropes et. al. Supplemental Figure 5





Supplemental Figure Legends

Supplemental Figure 1. Restriction mapping and Southern hybridization confirms the integrity of the US28/FL1-314 mutant. A) FIX-BAC DNA from the parental BAC, US28/WT, and US28/1-314 was digested with BglII, electrophoresed on a 0.6% agarose gel, and visualized by ethidium bromide staining. B) BglII digested BAC DNA was subjected to Southern blotting the blot was hybridized with a [^{32}P]-oligonucleotide probe internal to the US28 gene corresponding to nucleotides 23758-23788 in FIX-BAC. The probe recognizes only a single BglII fragment containing the US28 gene. The predicted molecular weight of this fragment is 14707 bp for the parental BAC, 14820 bp for US28/WT, and 14700 bp for US28/1-314.

Supplemental Figure 2. US28 directed stimulation of Ca^{2+} release requires the amino terminal ligand binding domain. HFFs were infected with US28/WT, US28/1-314, US28ΔN, or ΔUS28 viruses at an MOI of 3. At 48 hpi, the effects of CCL5 was measured by labeling cells with Fluo-4 AM and analyzing calcium signaling after addition of 10nM CCL5 using a FlexStation II Fluorometer. The caclium traces were followed out to 300 seconds (5 minutes) post addition of the CCL5. As observed in Figure 6, the US28/1-314 mutant protein exhibits an extended induction of Ca^{2+} release, consistent with its inability to undergo desensitization. While US28/WT signaling returns to baseline at approximately 80-100 seconds, US28/1-314 signaling returns to baseline at approximately 200-250 seconds. The results also demonstrate that that US28 induced calcium signaling requires the presence of the amino terminal ligand binding domain, as the US28/ΔN mutant which is deleted for the amino terminal 16 amino acids of US28 fails to induce any mobilization of calcium.

Supplemental Figure 3. Determination of the EC_{50} for CCL5 and CX3CL1 induced Ca^{2+} signaling through US28. HEK-293 cells stably expressing US28 were stimulated with increasing concentrations of CCL5 (upper panel) or CX3CL1 (lower panel) and calcium signaling was assessed by labeling cells with Fluo-4 AM and fluorescence measured using on a FlexStation II Fluorometer. Both CCL5 and CX3CL1 induces calcium signaling through US28 with and EC_{50} of 2nM.

Supplemental Figure 4. Agonist-independent US28 Stimulated Inositol Phosphate Accumulation Requires Gq/11 proteins. A) Cells transduced with the control retrovirus MIGR1 or the US28 expressing retrovirus MIGR1-US28 were labelled with 1mCi/ml [^{3}H]myo-inositol and accumulated inositol phosphates were isolated using anion exchange chromatography. The data represent at least six independent experiments performed in duplicate and are presented as a ratio of accumulated inositol phosphates to total [^{3}H] incorporation. B) Analyses of US28 expression. US28 was immunoprecipitated from cell lysates using M2-agarose beads and immunoprecipitates were blotted for FLAG-tagged US28 using an α-FLAG polyclonal antibody. GAPDH expression in whole cell extracts is shown for control purposes.

Supplemental Figure 5. US28 exhibits hyperactive signaling activity in βarrestin deficient cells indicating a failure to be regulated by the cellular desensitization machinery. A) Cells transduced with the control retrovirus MIGR1 or the US28 expressing retrovirus MIGR1-US28 were labelled with 1mCi/ml [^{3}H]myo-inositol and accumulated inositol phosphates were isolated using anion exchange chromatography. The data represent at least four independent experiments performed in duplicate and are presented as a ratio of accumulated inositol phosphates to total [^{3}H] incorporation. B) Analyses of US28 expression. US28 was immunoprecipitated from cell lysates using M2-agarose beads and immunoprecipitates were blotted for FLAG-tagged US28 using a a-FLAG polyclonal antibody. GAPDH and βarrestin expression in whole cell extracts is shown for control purposes.